UNIT 2 HOW CELLS ARE STUDIED ?

Structure

2.1 INTRODUCTION

In Unit 1, you learnt about the evolution of the cell and a historical account of the growth of cell biology. In this Unit, you will study about the various tools and techniques used to study the cell.

Cells, due to their minute size, cannot be observed by the naked eye. Lenses can magnify minute objects only upto a limited extent. Therefore, many lenses were combined together to form an instrument known as the microscope (Gr mikros—small, skopein—to see). Subsequently, many new tools and techniques were invented by cell biologists about which you will study in detail in this Unit.

We begin the Unit with light microscope (LM). Here, you will know about the difference between resolving power and magnification and various cytochemical techniques which are used to study the chemical components and structural organisation of the cell. Phase contrast microscopy; an important technique for viewing the live cells is described. You will also learn about electron microscopy (EM), the best known method for the study of the ultrastructure of the cell and an important technique called 'freeze fracturing' which is used to study the internal structure of membrane in the cell.

Use of radioisotopes, antibodies, and fluorescence microscopy for localisation of various cell components are discussed in this Unit. You will also learn about cell culture and cinemicrography, the other important techniques used to study live cells.

While going through this unit, it would be useful to recall and apply your knowledge of elementary chemistry, physics and mathematics, such as radioisotopes, refractive index and simple mathematical calculations. The study of this Unit assumes that you are familiar with the optical laws and principles on which a microscope works.

Objectives

After reading this Unit you should be able to:

- explain the working and usefulness of a light microscope and phase contrast microscope,
- list the principles and uses of fluorescence microscopy,
- suggest simple cytochemical methods to locate major cell components,
- explain the principles of transmission and scanning electron microscopy and their advantages and disadvantages as compared to light microscopy,
- explain the use of freeze fracture and cell culture techniques,

- suggest uses of cinemicrography in the study of cell,
- explain the uses of radioisotopes (with special reference to autoradiography) and antibodies for localising specific molecule in the cell.

2.2 LIGHT MICROSCOPY (LM)

Metric measurements of size

1 meter = 100 centimeter (cm) (M)

1 cm = 10 millimeter (mm)

1 mm = 1000 micrometer (µm)

1 µm = 1000 nanometer (nm)

1 nm = 10 Angstrom (A°)

A study of the structure of organisms is an essential requirement of biology. However, unaided human eye cannot see objects smaller than 0.1 mm(100 µ) in diameter. Therefore,various devices were discovered to increase his ability to observe minute structures. The knowledge of these devices is responsible for understanding structure and function of cell (Table 2.1).

Table 2.1 Some Important Landmarks in the History of Light Microscopy

Year	Nature of Discovery
1655	Small pores described in a section of cork, were named as cells by Hooke.
1674	Protozoa was discovered by Leeuwenhoek by the use of a microscope.
1835	Cell theory, i.e., nucleated cell is the unit of structure and function in all plants and animals, was proposed by Schleiden and Schwann.
1881	Use of staining techniques was introduced by Cajal and others.
1886	Lenses were made to study minute details of the structure of cell.
1898	Golgi apparatus was seen and described for the first time by Camillo Golgi by staining cells with silver nitrate.
1932	Phase contrast microscope was discovered to view live cells.
1941	Antibodies coupled to fluorescent dyes were used by Coons to detect cellular antigens.

The earliest microscopes had only one lens between the object and the eye and were, therefore, called **simple microscopes**. The modern microscopes, on the other hand have two lenses, the objective and the eye piece, in a series, to view an object. These are known as **compound microscopes** (Fig. 2.1).

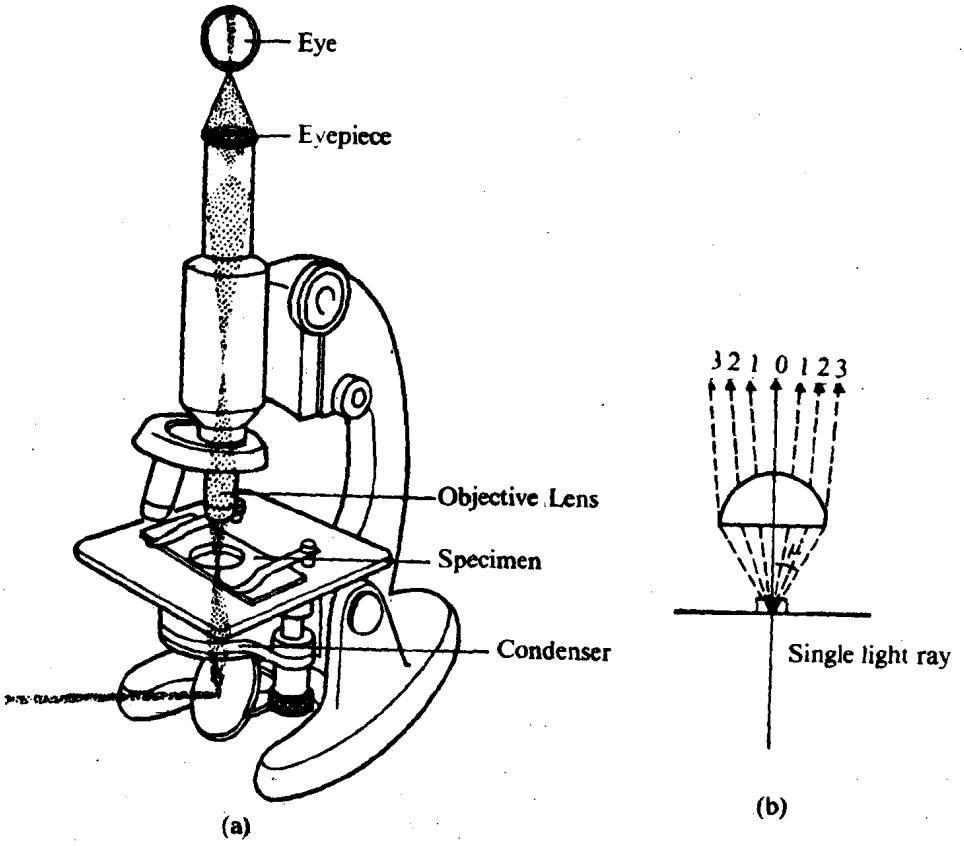

Fig. 2.1 : a) Light path in a compound microscope. The objective lens is near the specimen and the observer looks into the eye piece at the upper end of the tube of the microscope. The specimen is beyond the focal point of the objective lens.
b) A single light ray pattern.

With the help of a compound microscope one can view the objects at a very high magnification. However, the important property of a light microscope is not its magnifying power but its resolving power. Let us now explain the terms magnifying power and resolving power. The **magnifying power** of a microscope is its ability to show an enlarged or magnified view of an object to the eye. The resolving power, on the other hand, represents the ability of the microscope to show the fine details in the object to the eye (Fig. 2.2)

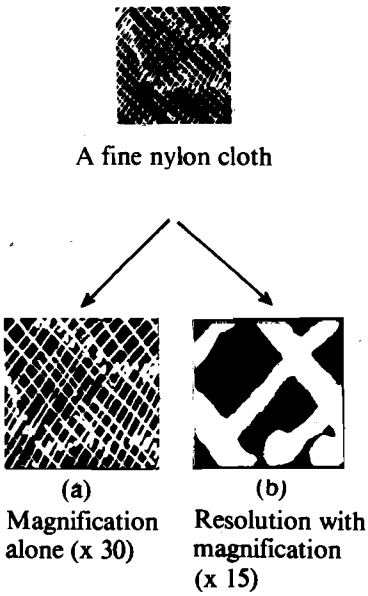

Fig. 2.2 : Magnification alone (a) the details of nylon cloth are not seen. Resolution with magnification (b) reveals that the cloth is made up of fine fibres.

When two objects are very close together, they may appear as one and when viewed through the microscope they may not appear as two separate objects. However, these two objects can be seen as two separate objects or in other words we can say that the two objects are just resolved if the microscope used is of high resolving power. The linear distance(s) between the two objects that is just resolved by the microscope is given by

Numerical Aperture (NA): A measure of the resolving power of a microscope objective, equal to the product of the refractive index of the medium (n) in front of the objective and the sine of the angle (sin α) between the outermost ray entering the objective and the optical axis.

$$s = \frac{0.61\,\lambda}{NA} \qquad \text{.... Equation 2.1}$$

(λ = wavelength of the light used
NA = numerical aperture of the objective of the microscope)

The minimum distance 's' is called as the **'limit of resolution'** of the microscope. Smaller the value of 's', higher is said to be the resolving power. The resolving power of a microscope depends on the numerical aperture of its objective. The greater the magnitude of NA, the smaller is the value of 's', that is, finer is the detail that can be observed.

To obtain better resolution with microscopes we often use ultraviolet light which has a smaller λ. The electron beams which behave like waves under some circumstances, have wavelengths 10^5 times shorter than visible light. This has led to the development of electron microscopes which have extremely high resolving power.

Thus, to increase the resolving power of a microscope or to diminish the distance 's' between the two points to be resolved by the microscope, we often use light which has a smaller λ and the objective whose aperture is large (see Equation 2.1).

Equation 2.1 shows that to obtain better resolution, light of smaller λ should be used e.g. ultraviolet light and even electron beams which behave like waves under some circumstances. Electron beams have wavelengths 10^5 times shorter than visible light. This has led to the development of electron microscopes which have extremely high resolving power.

Using the LM, we can study the cell by various methods. You will study about three such methods in the following sub-sections.

2.2.1 Cytochemistry

It is well known that certain stains and dyes specifically combine with certain chemical components of the cell. Cytochemical techniques take advantage of this fact and help in localisation and identification of various constituents like proteins, nucleic acids, lipids, etc. within a cell. For this a particular molecule is first immobilized and then identified by its reaction with a stain. Some molecules are also identified by their physical properties like absorption of radiation at a particular wavelength. For example, the nucleic acids absorb UV rays at 260 nm whereas the proteins absorb at 280 nm. You will learn more about the chemical components of the cell in Units 4 and 5.

The amount of the chemical component present in a cell can be quantified provided the reaction between the molecule present and the stain is strictly on 1: 1 basis, as in the case of Feulgen reaction for DNA described in Table 2.2.

A large number of dyes are in use to stain different chemical components of the cell. However, the chemical basis for the specificity of many dyes is not known, so only qualitative information can be obtained. The following table (Table 2.2) lists some of the common tests for the detection of proteins, lipids, nucleic acids and polysaccharides.

Table 2.2 : Some Reactions and Principles of Cytochemical Stains

Substance		Reaction or Test	Principle
1) Proteins	a)	Diazonium reaction (staining with diazonium hydroxide)	Detects tyrosine, tryptophan and histidine groups in the protein.
	b)	Fast green	Stains histone proteins.
	c)	Bromophenol blue	Stains all proteins.
2) Lipids	a)	Osmium tetroxide	Stains unsaturated fatty acids black.
	b)	Sudan black B	Causes black colour by dissolving in lipid droplets.
3) Nucleic acids	a)	Feulgen reaction	Acid hydrolysis exposes the free aldehyde groups in deoxyribose. The free aldehyde group is made to react with Schiff's reagent to produce a purple colour.
	b)	Methyl green pyronin (MGP)	Methyl green stains DNA while pyronin stains RNA. Both the dyes combine with phosphoric acid.
4) Polysaccharides		Periodic acid Schiff (PAS) reaction	Periodic acid oxidises 1, 2 glycol groupings to liberate free aldehyde groups which are stained purple by Schiff's reagent.

Hydrolysis: The cleavage of a molecule into two or more molecules by the addition of a wate molecule.

Glycol groupings i.e. $\begin{matrix} CH_2OH \\ | \\ CH_2OH \end{matrix}$

SAQ 1

Schiff's reagent is used to localise DNA as well as polysaccharides in the cell. But in both the reactions, its mode of action is same. Explain in about 6-7 lines in the space provided.

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2.2.2 Fluorescence Microscopy

Certain compounds when exposed to short wavelength radiations, e.g., UV and X-rays, absorb and emit energy as light of a longer wavelength. This process of emitting light from a compound is known as **fluorescence** and is now commonly used in microscopy. Compounds which naturally emit light exist in most living tissues. For example, the mitochondria of liver and kidney, collagen and chlorophyll give a strong fluorescence. Such a type of natural fluorescence is called as **autofluorescence**. Sometimes fluorescent dyes

like rhodamine, quinacrine etc. called **fluorochromes** are used to stain cell components to produce secondary fluorescence. Such important dyes are also used in the study of chromosome behaviour.

The fluorescent microscope is essentially an ordinary optical instrument. It has been modified by the incorporation of special filters. These filters allow only the required wavelength of light to pass and cause fluorescence in the specimen. The most significant application of fluorescent microscopy is in the field of immuno-fluorescent antibody labelling technique about which you will study later in this Unit.

2.2.3 Phase Contrast Microscopy

Phase contrast microscope has the resolving power similar to ordinary light microscope. However, it enables us to observe the cellular organelles in living cell. The working of this microscope is based on the principle of phase contrast.

Phase: The fractional part of a period through which the time variable of a periodic quantity has moved, as measured at any point in time from an arbitrary time origin, usually expressed in terms of angular measure, with one period being equal to 360°.

To understand phase contrast microscopy you should know that whenever two light waves interfere with each other, the resultant light wave may have either greater or lesser amplitude depending on the phases of the light waves (Fig. 2.3).

It is a known fact that a light wave passing through a transparent object suffers a change in phase.

The living cells are heterogeneous in their cellular components having different thickness. Due to this difference, each component has different refractive index. Hence, when a light wave passes through the cell, a change in phase is produced due to differences of thickness and refractive indices of cellular components (Fig. 2.4). The phase contrast microscope exaggerates the small difference between the phases and enables one to distinguish the adjacent structures.

Refractive index: The ratio of the phase velocity of light in a vacuum to that in a specified medium.

2.3 ELECTRON MICROSCOPY (EM)

In EM, the object is viewed by a beam of electrons instead of visible light. An electron beam has a shorter wavelength ($\lambda = 0.004$ nm) compared with visible light ($\lambda = 400$ nm). Therefore, an EM has higher resolving power than LM (Equation 2.1). Till now electron microscopy is the best available method to study biological ultrastructure (Fig. 2.5).

The resolving power of most modern EMs ranges between 100 μm to 0.1 nm. However, problems of specimen preparation, and radiation damage limit the resolution of biological objects to about 2 nm, even this is 100 times better than the resolution achieved by the best LMs. Further, the magnifying power of the EM is upto 200,000 X. The two types of EM are **transmission electron microscope (TEM)** and **scanning electron microscope (SEM)**. about which you will study in the following section.

2.3.1 Transmission Electron Microscopy

The source of illumination in a TEM is a tungsten filament that emits electrons. A vacuum is created to prevent the collision of electrons with anything else except the specimen. The electron beam is focused by different electromagnetic coils (lenses). First electrons are collected and focused in an electromagnetic 'condenser' lens and then they are collected again by an 'objective' lens. The electromagnetic objective lens produces a magnified image of the object which is received by 'ocular or projection' lens. The final image is formed either on a photographic plate or on a phosphorescent screen. The image results from differential scattering of electrons from molecular constituents of the cell.

If you compare the functioning of a TEM with a LM, you will observe that while there are many similarities between a LM and a TEM, there are also many differences (Fig. 2.6). The image formation in a LM depends on the degree of light absorption whereas in a TEM it depends on electron scattering. The lenses of LMs are glass lenses and focusing is done by moving them nearer or farther away from the specimen. In TEMs all the lenses are electromagnetic coils and focusing is done by manipulating the amount of current flowing through the magnetic coils. The final image in a LM is seen by the eye whereas in a TEM it is formed on a phosphorescent screen.

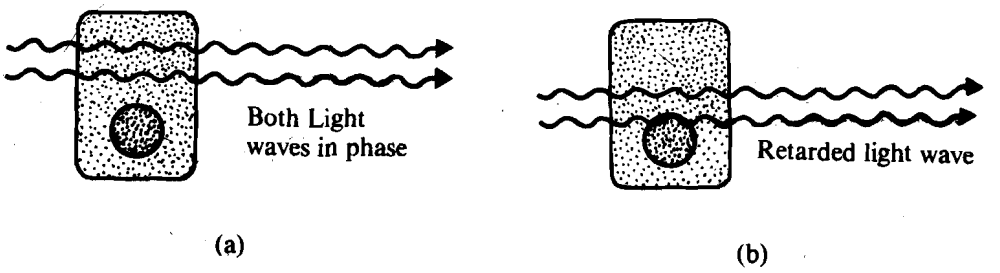

Fig. 2.3 : a) When two light waves are passing side by side, through a cell and if they have encountered the same refractive index and thickness of medium, they will emerge in phases.
b) When one light wave passes through an organelle which is not passed through by other, then it will be retarded and will emerge slightly out of phase.

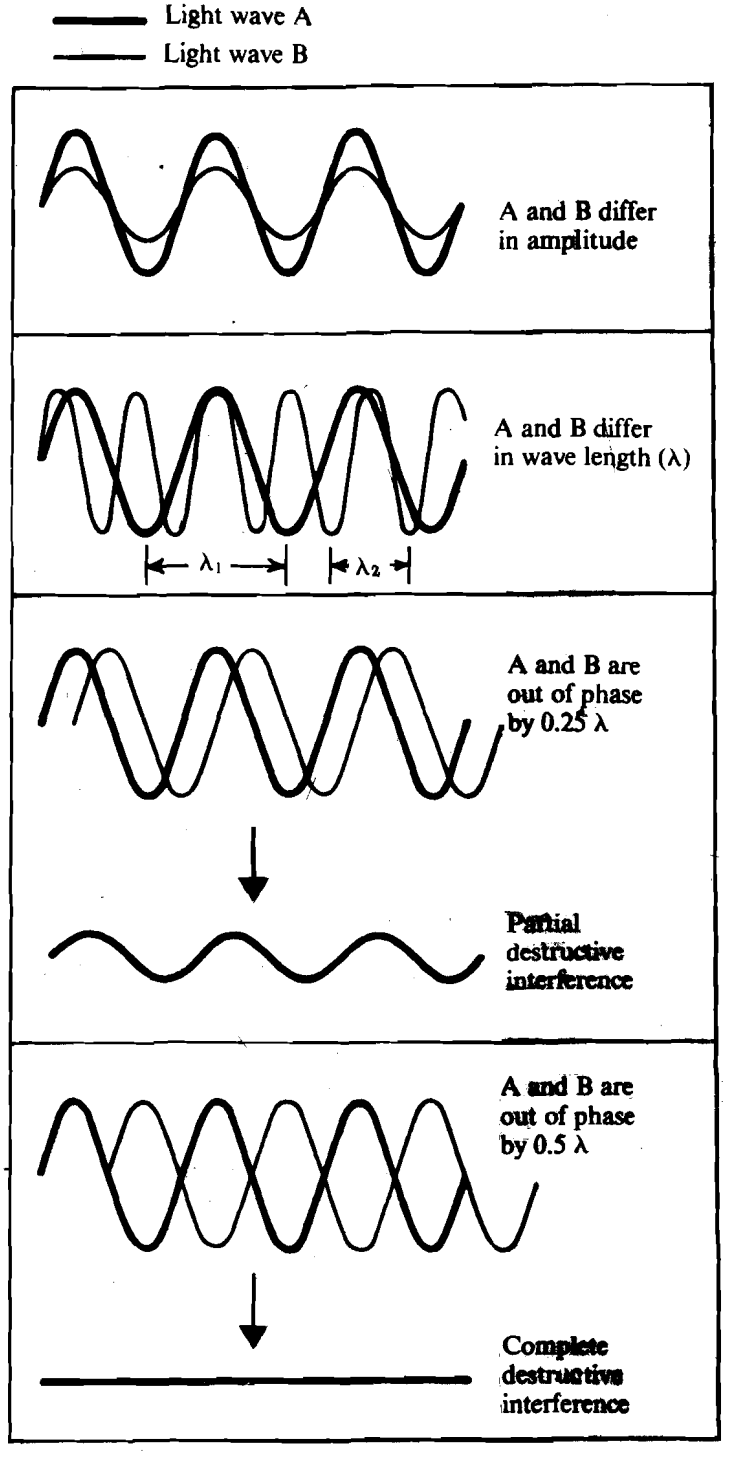

Fig. 2.4 : Properties of light waves that result in partial and complete destructive interference.

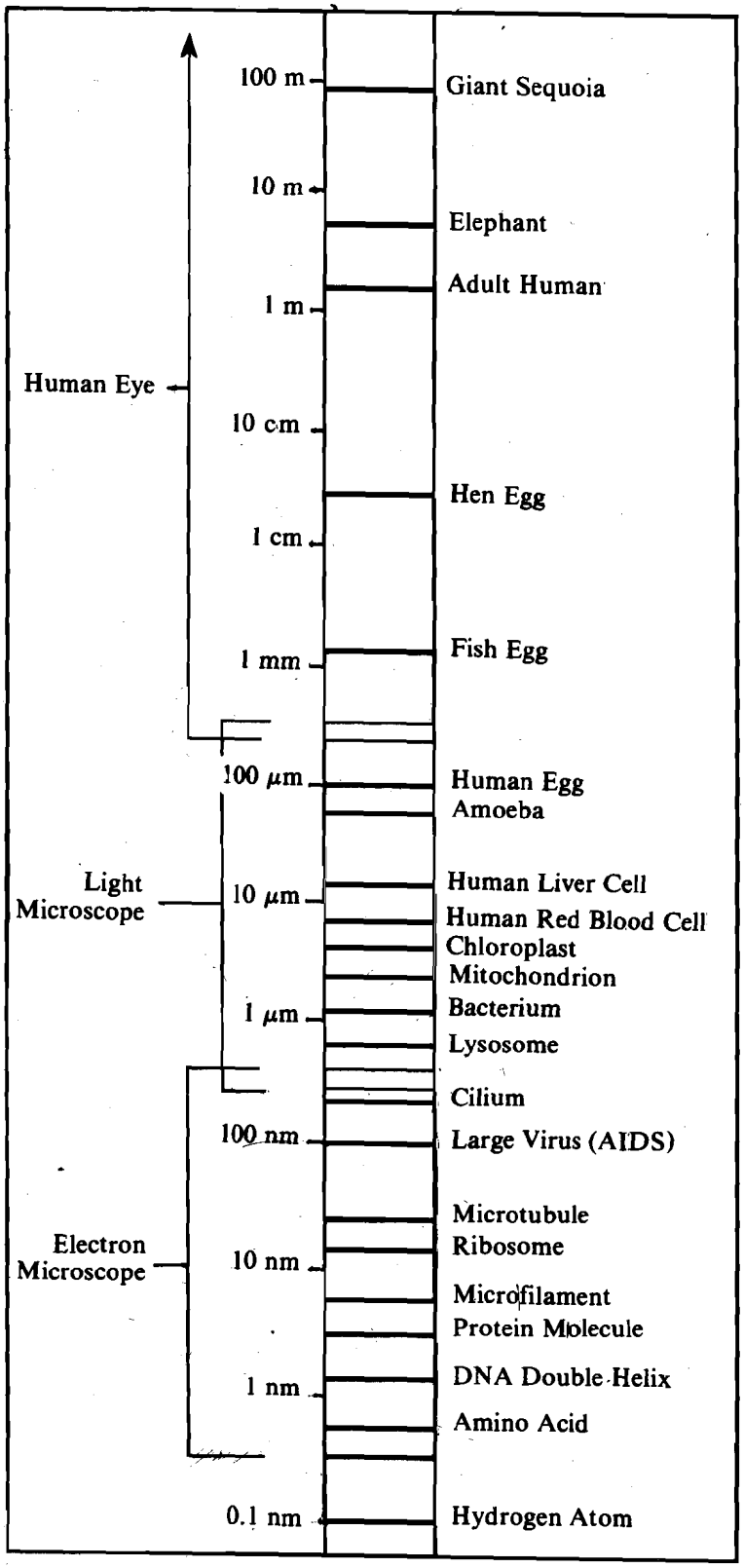

Fig. 2.5 : Logarithmic scale of microscopic dimensions. The relationship between resolving power of human eye, the light microscope and the electron microscope with sizes of various biological structures like bacteria, virus etc. is shown. Bacteria are generally 1 to 2 micrometers (μm) thick, and human cells are of larger magnitudes. The scale goes from nanometers (nm) to micrometers (μm) to millimeters (mm) to centimeters (cm) and finally meters (m)

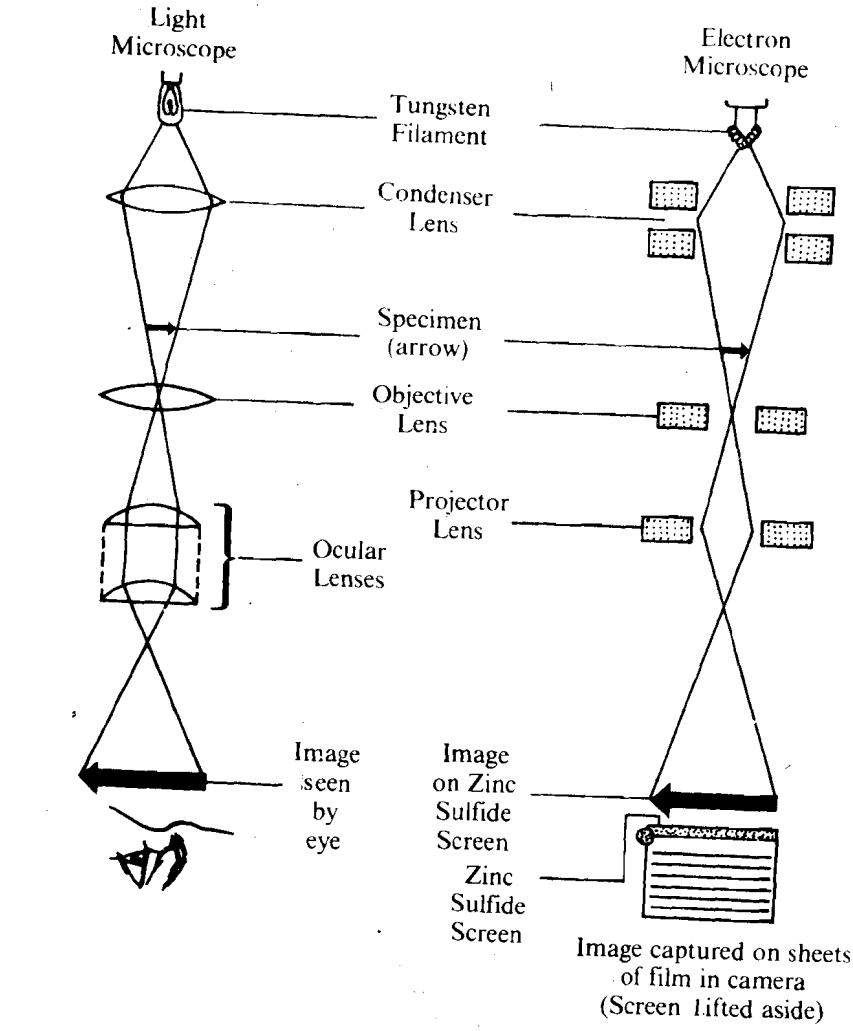

Fig. 2.6 : a) A comparison of the component parts of the light microscope and electron microscope. b) A comparison of a transmission electron microscope and a scanning electron microscope. (TEM and SEM) Both require the specimen to be placed in a vacuum. In both, the electrons are accelerated from the filament and passed through a tiny hole to form an electron beam. In both, the beam passes through different magnetic coils, but in a TEM the image is viewed on fluorescent screen whereas in a SEM, the image is viewed on viewing screen.

An EM has the major advantage of increased resolution over a LM. The applications of electron microscope to cell biology are given in table (Table 2.3). Moreover, TEM has its disadvantages which are (i) the live biological material cannot be observed since electron beams traverse in vacuum, and (ii) only very small areas of tissues can be viewed at a time. Therefore, a LM and a TEM are complementary to each other.

Table 2.3
Major Events in the Development of the Electron Microscopy

1926	**Busch** proved that it was possible to focus a beam of electrons with a cylindrical magnetic lens, thus laying the foundations of electron optics.
1939	**Siemens** produced the first commercial transmission electron microscope.
1952	**Palade**, **Porter**, and **Sjostrand** developed methods of fixation and thin sectioning to observe many intracellular structures.
1953	**Porter** and **Blum** developed the first widely accepted ultramicrotome.
1957	**Steere** developed freeze fracture techniques which were later perfected by **Moore** and **Muhlethaler**.
1965	First commercial scanning electron microscope produced.
1986	**de Rosier** and **Klug** described techniques for the reconstruction of three dimensional structures from electron micrographs.

2.3.2 Scanning Electron Microscopy (SEM)

While TEM is useful to study the internal structure of the cell, SEM is an important technique if you wish to examine the surface features of the specimen. Moreover, a specimen can be viewed at different angles. The resolving power of a SEM is lower than that of a TEM since a resolution of only about 3.0 nm can be attained by SEM.

In a SEM, a low energy beam of electrons (primary electrons) strikes the surface of a specimen. This causes the emission of the secondary electrons from the specimen which are gathered by a photomultiplier tube to form an image which is built up line by line as in the case of television. The image formed by SEM has a three-dimensional appearance. Unlike in a TEM, the electrons in a SEM do not pass through the specimen.

2.3.3 Freeze Fracture Electron Microscopy

Freeze fracture electron microscopy is a technique to reveal the surface features of hydrophobic interior of cell membranes. In this technique, the cells/tissues are first frozen in liquid freon at – 130°C. The frozen block is cut with a cold knife blade alongwith the planes of weakness, run through each cell. Generally, these planes occur between the two layers of lipid molecules of the membrane. The plane of fracture at these places exposes the interior of a plasma membrane (Fig. 2.7).

The newly exposed faces of a plasma membrane, the exoplasmic fracture face (EF) and the protoplasmic fracture face (PF) are covered with a very thin layer of platinum by dissolving away the organic material. The freshly fractured membrane may be covered with platinum and carbon to obtain replicas (exact copies) of the surface and this is observed under a TEM.

This technique demonstrates that intercellular junctions (where cells make contact with each other) are composed of specialised membrane proteins.

SAQ 2

Which microscope (LM, TEM or SEM) will you use in the following cases. Give your answers in the space provided.

If you want to :

a) observe details of the surface of an insect's compound eye.

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b) examine the patterns of capillaries in a section of skin.

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c) examine a kidney cell to look at its cell membrane.

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d) identify the type of flagellum of a flagellate organism.

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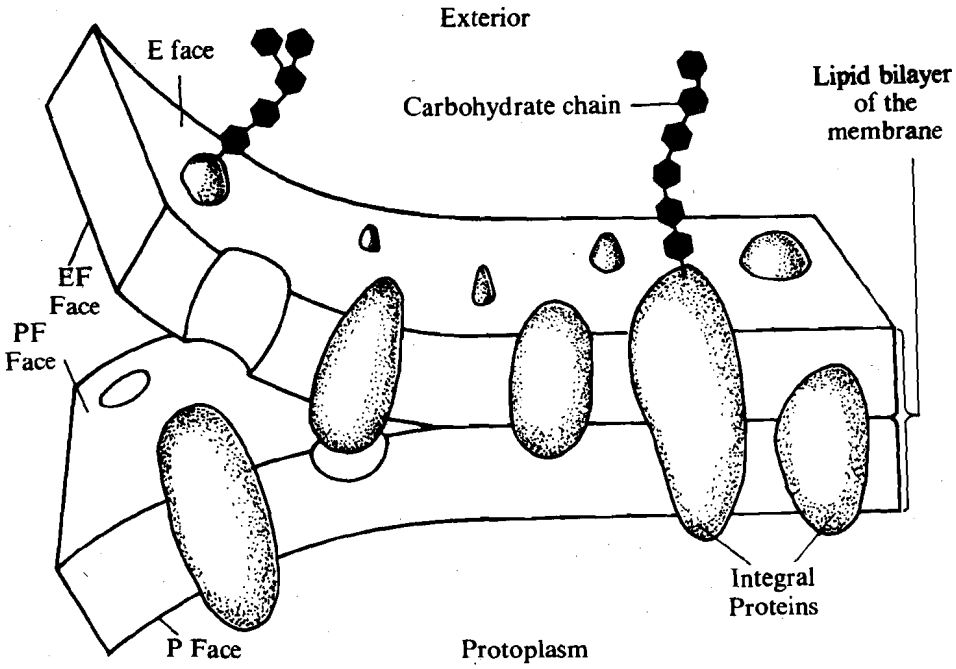


Fig. 2.7 : Tissue is fractured by using a microtome knife. When the plane of fracture intersects the cell membranes, e.g., plasmalemma, nuclear envelope, etc. the membrane is split to produce two half membranes: E (exterior) half and P (protoplasmic) half. The surface features of the fracture faces that are produced reveal the location of proteins.

2.4 RADIOISOTOPES, AUTORADIOGRAPHY AND ANTIBODIES

Isotopes: Isotopes are chemical elements that have the same atomic number (e.g. the number of protons in the nucleus of the atom) but different atomic weight (i.e. total number of protons and neutrons in the nucleus).

Radioisotopes are those forms of an element which exhibit radioactivity and so can be used as markers or tracers. For this purpose, we first introduce the radioisotope in a molecule in the cell and then detect the radioactivity with the help of certain device. For example, if we replace a suitable hydrogen atom of thymidine by ^{3}H, we may trace the course of thymidine in the cell. Since thymidine is a component of DNA but not of RNA, we can be sure that we are following only DNA metabolism. Similarly, to know the course of protein synthesis we may use radioactively labelled amino acids. Many of the commonly used radioactive isotopes are listed in table (Table 2.4).

Table 2.4
Some Radioisotopes Frequently Used as Tracers

Isotope	Used as a Tracer of
^{3}H	Virtually any organic compound
^{14}C	Virtually any organic compound
^{24}NA	Salt metabolism, exchanges across membranes
^{32}P	Nucleic acid metabolism, phospholipid metabolism
^{35}S	Protein metabolism
^{36}Cl	Salt metabolism
^{45}Ca	Salt metabolism, bone deposition
^{59}Fe	Heme synthesis

Radioisotopes are used to (a) measure the rates of metabolic turnover of a material within a cell or tissue, (b) locate the site of synthesis of molecules, (c) measure the rate of exchange of materials across cellular membranes, (d) locate a molecule in a cell. Radioisotopes are widely applied to study also **the precursor-product relationship. Many times when a radioactively labelled molecule (precursor) is introduced into a system, the molecule is chemically converted into another form (product). For example, when radioactive iron is** introduced in blood, radioactivity can be detected in liver ferritin and haemoglobin of red blood cells. Similarly, radioactivity of ^{32}P — labelled phosphate can be detected in many phospholipids.

There are two methods to assay the incorporated radioisotope.

Bioassay: A test that uses living organisms rather than chemical analysis to detect the presence of substances such as hormones, which are usually present in very small quantities and are hard to detect by chemical means but easy to detect from their effect on an organism's behaviour or physiology.

i) The radioactive molecule is introduced in the cell either *in-vivo* or *in-vitro*. The cell incorporates and metabolises the radioactive molecule and after a certain time interval radioactivity can be detected and measured with the help of the special instrument routinely known as counters. The selection of instruments is based on the type and energy emitted by the radioactive substance. The most commonly used detectors are **Geiger Muller** counters commonly known as GM counters, **solid scintillation counters** and **liquid scintillation counters**. GM counters are generally used for isotopes emitting beta radiations of intermediate or high energy waves. These are generally used in tracer experiment involving ^{24}Na and ^{32}P. Liquid scintillation counters are used for isotopes emitting low energy beta radiations. These counters are used for tracing ^{3}H and ^{14}C. Solid scintillation counters are most commonly used for isotopes emitting gamma rays, for example ^{131}I.

ii) Autoradiography is a modification of the radioactive tracer technique. In this technique, the radioactivity is detected by a thin film of photographic emulsion containing silver compounds which is put over a labelled cell or tissue. The rays emitted by radioisotope from the cell or tissue expose the photographic emulsion like a photographic film which is developed in a dark room. Silver grains are seen as black dots on parts of the specimen where radioisotope is accumulated.

Autoradiography reveals the metabolic processes of the cell. For example, by labelling cells with ^{3}H thymidine, it is found that silver grains are localised in nucleus, which shows that DNA is synthesised in the nucleus (Fig. 2.8). Similarly, by labelling cells with ^{3}H uridine, silver grains are first seen in nucleus and then after some time these grains appear in the cytoplasm. This clearly demonstrates that RNA is initially synthesised in the nucleus but later on migrates to the cytoplasm.

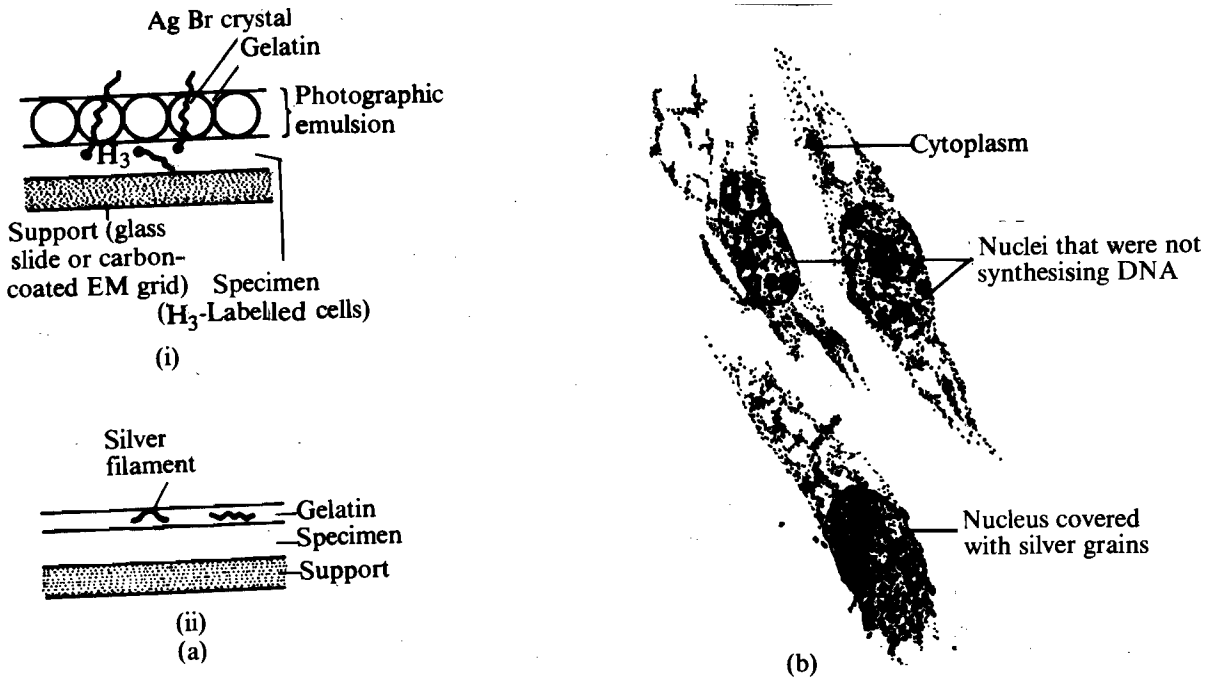


Fig. 2.8 : a) The technique of autoradiography.
Cells containing a suitable radioactive isotope after a time interval are attached to a support (glass slide in case of light microscopic autoradiography). The photographic emulsion (containing silver salts such as silver bromide) forms a layer over the cells. The regions of cells containing labelled molecules emit radioactive particles which sensitize the salts of the emulsion. The photographic emulsion is developed. The unexposed salts are dissolved and washed away. The silver grains show the location of labelled molecules in the cell.
b) An actual autoradiograph. The most commonly used tracers are ^{14}C and ^{3}H, as both are common constituents of organic molecules and do not involve high risk in handling by the scientists since they emit very weak beta particles.

Different radioisotopes are used for localising different macromolecules. As stated earlier, cells are labelled with ^{3}H thymidine for the study of DNA metabolism, ^{3}H uridine to study RNA metabolism, tritiated amino acids for examining the synthesis of proteins, tritiated monosaccharides such as ^{3}H-mannose and ^{3}H-fucose for examining the synthesis of polysaccharides.

Antibodies : Antibodies are proteins that bind tightly and specifically to the antigens, i.e., antibody producing agent. Animals produce antibodies in response to an invasion by an infectious agent which acts as an antigen. Antigen causes an organism to make a larger number of different antibody proteins. Binding of an antibody with the antigen allows certain white blood cells to recognise the foreign substance and degrade it. Thus, antibody acts as a signal for the removal of infectious agents.

Antibodies

Antibodies are important tools for detecting and localising specific molecules in the cells due to their high specificity. The first requirement for this is to produce antibodies against a specific molecule. Suppose you want to produce antibodies against tubulin, the purified tubulin is first obtained from a given tissue. It is then injected into an animal such as rabbit which in response to tubulin produces antibodies. Blood sample is collected from the animal and antibodies are isolated. These antibodies are used to localise the specific molecule in the cell because they bind only to an antigen, which is a tubulin in this case. Localisation can be done by two methods: direct and indirect.

In the direct method, antibodies are first labelled with a fluorescent dye-like rhodamine for light microscopy, or with a high molecular weight, electron dense compound like ferritin for transmission electron microscopy. Fluorescent labelled antibodies are allowed to react with cells so as to form specific antigen-antibody complex. The complex is then examined under a microscope (fluorescent microscope or TEM). The specific molecules will emit light or become electron dense due to their binding with labelled antibodies (Fig. 2.9). This method is used to localise foreign bodies such as viruses, bacterial antigens, etc.

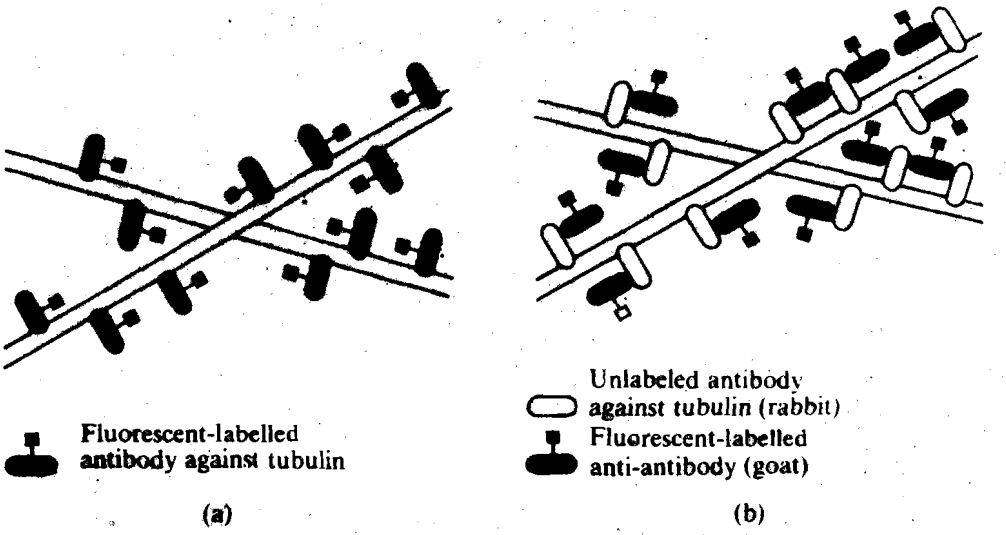
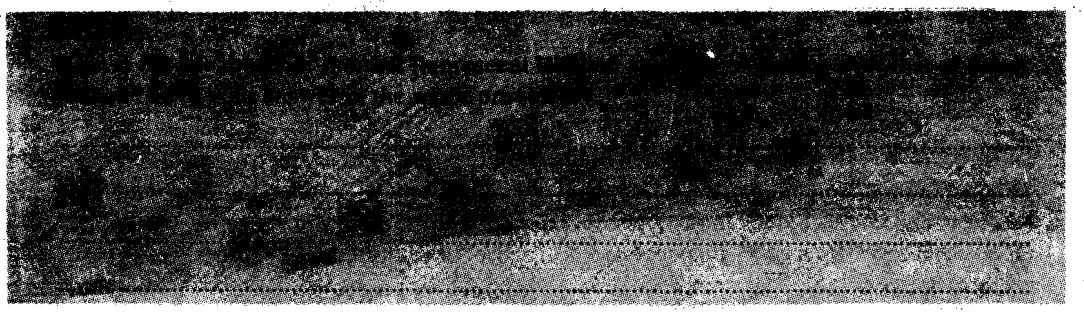


Fig. 2.9 : a) Direct fluorescent labelled antibody technique.
Tubulin antibodies attach only to microtubules, since they contain tubulin. The location of microtubules in the cell becomes evident, due to the fluorescence label of the antibody
b) Indirect fluorescent labelled antibody technique.
Two types of antibodies are used in this technique. The first one is produced in rabbit, against tubulin. It is not labelled with fluorescent material. The second antibody is produced in goat against the first type of antibody. The goat antibodies against rabbit antibodies are labelled with a fluorescent dye. The rabbit antibodies are sandwiched between antigen (tubulin) and labelled antibodies (goat antibody).

In the indirect method, two types of antibodies are used. The first antibody against tubulin is produced in the same way as described above. Howevér, this antibody is not labelled. The second antibody is produced in another animal (it may be a goat), against the first antibody (rabbit antibodies). The second antibody binds only to the rabbit antibodies. The second antibody is labelled either with fluorescent dye or with ferritin. On reaction, the tubulin antibody (produced in rabbit) combines with the tubulin on the one hand and with the antirabbit antibody (produced in goat) on the other.

The advantage of the indirect method over the direct technique is that only one type of antibody containing a fluorescent label need be prepared. This one preparation can be used to light up any cell component that has been reacted with its homologous antibody.



2.5 TISSUE CULTURE AND CINEMICROGRAPHY

Tissue culture and cinemicrography are two important techniques which have helped greatly in the advancement of cell and molecular biology. Tissue culture helps in maintenance of cells alive outside the living organisms (plants and animals). Cinemicrography helps in following the events, specially slow events like cell division or cell locomotion. You will study about these two techniques in detail in the following sub-sections.

2.5.1 Tissue Culture

It is an important technique for maintaining a part or piece of animal or plant tissue alive after their removal from the organism. To maintain the living cell outside an organism, the tissue pieces or cells are kept usually in culture dishes. It is necessary to provide an environment in which cells can live and multiply over a period of time. Therefore, different media containing mixture of salts, amino acids, vitamins, growth factors etc. are used for this purpose. There are some special types of culture media such as Balanced Salt Solution (BSS), and Essential Minimal Medium (EMM).

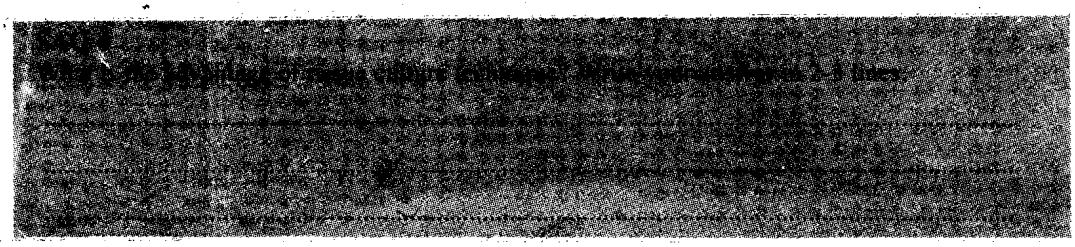
Cultures prepared directly from the tissue of an organism are called **primary cultures**. For obtaining the primary cultures, the organ is removed in a sterile atmosphere so as to make it free from bacteria and other foreign bodies. Then it is cut into small pieces and treated with trypsin. Trypsin has the property of dissociating cell aggregates into a suspension of single cells, without affecting their viability. Cultures prepared from cells which were removed from primary cultures form **secondary cultures**. Such cells show the properties of the parent body from which they were obtained, for example, fibroblasts secrete collagen; cells derived from embryonic skeletal muscle form muscle fibres. These phenomena which otherwise cannot be studied in intact tissues are easily studied by this technique.

Some cells show prolonged growth and divide indefinitely *in vitro*. These cells are called 'variant cells'. Such cells grow or propagate in a linear order indefinitely to form a cell line. Some commonly used **cell lines** are BHK 21 and Human HeLa cells which are derived from fibroblast of a hamster and epithelial cells of man respectively.

Many of these cell lines were derived from tumor like growth. All these cells are capable of dividing indefinitely in tissue culture and they express at least some of the properties which are different from the cell of origin. BHK 21 cells, and HeLa cells, are capable of growing in suspension whereas other cell lines require a solid culture substratum for their growth.

Unlike variant cells, cancer cells divide indefinitely *in vivo* (within the living organism) as well as *in vitro* (in the experimental conditions). They grow rapidly compared to normal cells in a tissue culture dish.

Tissue culture is an important technique which is used to study the fundamental problems in cell biology. One of the major advancements in tissue culture is **cloning**. A clone is a population of cells derived from a single parent cell.



2.5.2 Cinemicrography

Earlier it was impossible to follow the events within the cell due to:

i) non-visibility of cell organelles under a light microscope,

ii) maintenance of cells outside the body, and

iii) slow nature of events, like cell division or cell locomotion. It was not possible earlier to follow the events within the living cells. The first difficulty was overcome by the development of phase contrast microscopy and second was removed by the tissue culture technique. The third problem was solved with the onset of cinemicrography.

In cinemicrography the events in or about cells are continuously filmed by a camera attached to the eye piece of a microscope. The speed of exposure per unit time can be varied according to requirement. By an appropriate combination of the speed of exposure and the speed of viewing the film it is possible to see events that took 1 hour in just 1 min or 5 sec. This is very useful in making the observation on mitosis, cytokinesis, cell movement etc. On the contrary, in other cases, fast events can be slowed down for viewing, for example, the movement of cilia or flagella.

2.6 SUMMARY

To sum up, this unit dealt with the following:

- Observation of cellular structures is difficult because of their small size and lack of contrast in their components. These difficulties are overcome by the use of various tools and techniques such as light microscope and electron microscope. Cytochemistry deals with the identification and localisation of chemical components of the cell.
- Fluorescence microscopy is the technique by which components are recognised by the fluorescence they emit in the visible spectrum. Phase contrast microscopy is used for the study of living cells which are otherwise transparent to light.
- Electron microscopy (EM) is the best method for studying biological ultrastructure. The two types of EM are, transmission electron microscope (TEM) and scanning electron microscope (SEM).
- Freeze fracturing is a technique used to study the structure of membranes (plasmalemma, nuclear membrane etc.).
- Radioisotopes are used as tracers, to follow the course of a molecule or its metabolic pathways. In autoradiography substances labelled with radioisotopes are used. Like radioisotopes, antibodies are also used for localisation of macromolecules.
- Tissue culture is an important technique used to study live cells outside the parent body under suitable conditions.
- Likewise cinemicrography is another technique used to observe the flow of events within the living cells.

2.7 TERMINAL QUESTIONS

1) How can the resolving power of a microscope be increased? Give your answer in about 30 words.

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2) What are the applications of freeze fracturing? Write your answer in the space provided.

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3) Write two important uses of radioactive tracers in cell biology in the space given below.

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4) What is the difference between primary and secondary cell culture?

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2.8 ANSWERS

Self-assessment Questions

1) In the two reactions (Felugen and PAS), the first step is the exposure of free aldehyde groups of sugars (DNA, polysaccharides). This step is differently achieved in the two reactions. However the second reaction, that is, reaction of the free aldehyde group with Schiff's reagent to produce purple colour is common.

2) (a) SEM (b) LM (c) TEM (d) TEM

3) In indirect fluorescent labelled antibody technique, only one type of labelled antibody is required to detect and localise any antigen.

4) Tissue culture technique helps to observe cell/tissue of an organism in a suitable outside environment.

Terminal Questions

1) Resolving power of a microscope can be increased by using light of shorter wavelengths or increasing the numerical aperture of the objective lens (see Equation 2.1).

2) Freeze fracturing enables a study of the interior of cell membranes and membrane function.

3) Radioactive tracers are used to:

   a) locate the site of synthesis of various molecules,

   b) measure the rate of metabolic turnover of a material within the cell.

4) Primary cultures are obtained directly from an organism and secondary cultures are obtained from primary culture.